

A critical examination of the sodium dodecyl sulfate (SDS) sedimentation test for wheat meals

Craig F Morris,^{1*} Bozena Paszczynska,¹ Arthur D Bettge¹ and Garrison E King²

¹USDA-ARS Western Wheat Quality Laboratory, Washington State University, Pullman, WA 99164-6394, USA

²Department of Food Science and Human Nutrition, Washington State University, Pullman, WA 99164-6394, USA

Abstract: Sedimentation tests have long been used to characterise wheat flours and meals with the aim of predicting processing and end-product qualities. However, the use of the sodium dodecyl sulfate (SDS) sedimentation test for durum wheat (AACC International Approved Method 56–70) has not been characterised for hexaploid wheat varieties with a diverse range of protein quality and quantity. This paper reports the variation associated with important method parameters: sample weight, SDS concentration, technician, grinder and screen aperture (particle size). Sedimentation volumes were recorded every 5 min for 30 min and expressed as specific volume, i.e. sediment volume in mL g⁻¹ meal. Ten diverse hexaploid wheat samples of markedly different protein quality and quantity were examined. The SDS sedimentation assay was shown to be highly robust and reproducible, with ANOVA (analysis of variance) model *R*² values greater than 0.98 (individual time points). The procedure delineated soft and hard hexaploid wheat samples based on a combination of protein quantity and quality. Sample weight (if corrected to unit weight basis), recording time of at least 10 min, SDS stock concentration of at least 10 g L⁻¹ (final), grinder type and screen aperture were minor sources of variation in SDS sedimentation volume relative to the effects due to differences among wheat samples. Interactions among ANOVA model terms were of relatively minor importance.

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Keywords: SDS sedimentation; sodium dodecyl sulfate; wheat; quality tests; protein quality

INTRODUCTION

Sedimentation tests have long been used to characterise wheat (*Triticum aestivum* L. and *Triticum turgidum* ssp. *durum* (Desf.) Husn.) flours and meals with the aim of predicting processing and end-product qualities. The USDA-ARS Western Wheat Quality Laboratory is developing an automated, market-applicable sedimentation test with potential for both official and unofficial inspection of wheat. Herein we report the first part of that work, a critical examination of the sodium dodecyl sulfate sedimentation test for bread wheat meals.

The first landmark paper describing a sedimentation test for wheat was that of Zeleny.¹ As Zeleny¹ then stated, 'The United States Department of Agriculture is carrying on several lines of research in an effort to devise a suitable practical test that may be used in connection with the official inspection of wheat and that will reflect with at least reasonable reliability the baking quality of the flour that can be milled from the wheat'. The basis for the 'Zeleny method' (as it came to be known) was a body of research showing that wheats of different baking qualities produced flours and meals that differed in their gluten swelling, viscosity and settling (sedimentation) rates in acidulated water (reviewed by Zeleny¹). As

the Zeleny method became more widely used and studied, modifications were introduced to address poor visual discrimination between sediment and supernatant.^{2–5} All these methods used milled flour. Further modifications reduced the sample size.^{5–7}

McDermott and Redman⁸ (The original method was reported as 'McDermott EE and Redman DG, Small-scale tests of breadmaking quality. *FMBRA Bull No. 6*, Flour Milling and Baking Research Association, Chorleywood, Rickmansworth, Herts, UK, pp. 200–213 (1977)'). The FMBRA is now part of Campden and Chorleywood Food Research Association Group, Chipping Campden, Glos, UK. The report is proprietary and is neither widely available nor widely cited.) introduced the use of the detergent sodium dodecyl sulfate (SDS, a.k.a. lauryl sulfate). Their method involved the hydration of 6 g of meal with 50 mL of water in a 100 mL graduated cylinder ('rapid shaking' to mix), followed by the addition of 50 mL of SDS (~10 g L⁻¹ final concentration), mixing by inverting, adding 1 mL of diluted lactic acid (LA, ~0.94 g L⁻¹) and again inverting to mix. Sedimentation volume was recorded after 20 min of

* Correspondence to: Craig F Morris, USDA-ARS Western Wheat Quality Laboratory, E-202 Food Science and Human Nutrition Facility East, PO Box 646394, Washington State University, Pullman, WA 99164-6394, USA

E-mail: morrisc@wsu.edu

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settling. The study used a KT mill[†] that was supplied with the falling number apparatus. In their subsequent report⁹ the SDS and LA were added as 50 mL of an SDS/LA reagent. A Tecator Cyclotec mill (previously UDY Cyclone mill) with a 1 mm aperture screen was used to prepare meals. In their report the authors state that ‘... the Zeleny method does not work satisfactorily on whole meals’, whereas they indicated that the SDS sedimentation test worked equally well on meals and milled flours.

Blackman and Gill¹⁰ were among the first to apply the SDS sedimentation test of Axford *et al.*⁹ to bread wheat breeding populations. Quick and Donnelly¹¹ and Dexter *et al.*^{12,13} applied the SDS sedimentation test to durum wheats. Preston *et al.*¹⁴ applied the SDS sedimentation test to Canadian bread wheats and found that the sample weight had to be reduced from 6.0 to 4.5 g and the final swelling time from 20 to 15 min, since the strong, high-protein CWRS (Canadian Western Red Spring) samples gave sedimentation volumes too large for satisfactory discrimination (data were not shown). Dick and Quick¹⁵ studied the effect of reducing the whole meal durum wheat sample weight and varying other method parameters, including the SDS concentration. Kovacs¹⁶ also varied the SDS concentration for durum wheats.

McDonald¹⁷ reported on the work of the AACC Committee on Quality Tests for Wheat and Flour to develop a standardisation of the SDS sedimentation test for durum wheat. This method, which became Approved Method 56–70, reduced slightly the LA concentration. A final SDS concentration of 14.7 g L⁻¹ was selected. Grinding rate was varied in a Cyclone mill and found to have an effect on sedimentation volume. Slower grinding rates produced higher sedimentation volumes. The effect was suggested to be due to differences in particle size distribution.

Lorenzo and Kronstad¹⁸ used five hexaploid varietal wheat samples to judge the effects of SDS concentration, LA concentration and settling time. They reported that the best discrimination between three good and two poor loaf volume lines was obtained with 20 g L⁻¹ final SDS concentration and a 30 min reading time.

Krattiger and Law¹⁹ reported no time-dependent interaction effects on sedimentation volume (no differential effects on sedimentation rate). Small changes in SDS concentration and reductions in sample weight had no effect. Silvela *et al.*²⁰ found that the sediment volume of a high-sedimentation-volume sample remained nearly constant over a 24 h period, whereas that of a lower-sedimentation-volume sample decreased substantially. Baik *et al.*²¹ modified the SDS sedimentation test based on the Zeleny FY test. The

primary feature of their test included obtaining nine sediment volume readings, three each after three mixing times. Carter *et al.*²² examined the use of a modified micro sedimentation test^{15,23} for soft white and club wheat breeding and reported that sediment volumes were highly dependent on sample weight (0.35–0.80 g) and that the response to weight varied among varieties and different protein concentrations. Numerous other minor variations on the generalised Axford *et al.*⁹ method have been used.^{24–26}

AACC International has five Approved Methods that relate to wheat sedimentation tests. Approved Methods 56–60 (‘Sedimentation test for flour’), 56–61A (‘Sedimentation test for wheat’), 56–62 (‘Modified sedimentation test for wheat’) and 56–63 (‘Micro sedimentation test for wheat’) do not use SDS. Currently the only Approved Method that does use SDS is 56–70 (‘Sodium dodecyl sulfate sedimentation test for durum wheat’), which was first approved in 1984.¹⁷

Although the SDS sedimentation test has been widely used and modified, there is a paucity of detailed evaluations of the test parameters using bread wheat varieties of diverse protein content and quality. The research reported here critically examines the variation in SDS sedimentation volume over time of whole meals prepared from a series of hexaploid wheat varietal samples that vary in protein content and quality (gluten strength). Method variables analysed include SDS concentration, sample weight, technician, grinder and particle size.

MATERIALS AND METHODS

Wheat samples

Wheat samples were selected *a priori* to encompass a wide range of protein quality and quantity. The samples are listed in Table 1 and included: ‘Eltan’, a soft white winter (SWW); experimental line ‘EST03005’, a hard red spring (HRS) with Canadian

Table 1. Hexaploid wheat variety sample, location, protein content, market class and high-molecular-weight (HMW) glutenin subunit composition (A and D genomes) of whole-meal samples used to examine Approved Method 56–70, SDS sedimentation test for durum wheat

Sample	Location	Protein (g kg ⁻¹)	Class ^a	HMW subunit composition
Eltan	Bickleton	77	SWW	1, 5 + 10
Eltan	Pullman	90	–	–
EST03005	Lind	151	HRS	2*, 5 + 10
Estica	Bickleton	80	HRW	Null, 2 + 12
Estica	Lind	129	–	–
Estica	Pullman	90	–	–
Finley	Bickleton	94	HRW	1, 5 + 10
Finley	Lind	145	–	–
Finley	Pullman	100	–	–
Hiller	Bickleton	87	Club	Null, 2 + 12

^a SWW, soft white winter; HRS, hard red spring; HRW, hard red winter.

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Western Extra Strong varieties Bluesky and Wildcat in its pedigree ('Jefferson'*2/'Bluesky'/'Wildcat'); 'Estica' and 'Finley', hard red winters (HRW); and 'Hiller', a soft white winter Club. These samples, except EST03005, were obtained from the Washington State University Variety Testing Program. All samples were harvested in 2003 (winter wheats planted in autumn 2002) in Washington State at the following locations: Eltan at Bickleton and Lind; EST03005 at Lind; Estica at Bickleton and Pullman; Finley at Bickleton, Pullman and Lind; and Hiller at Bickleton.

Basic SDS sedimentation protocol

The basic SDS sedimentation protocol employed here followed Approved Method 56–70,²⁷ which uses 100 mL graduated cylinders, a stock solution of 30 g L⁻¹ SDS in ~0.012 mol L⁻¹ lactic acid, and 6.30 g of ground whole meal (see Ref. 27 for specific mixing regimen). Grain samples (~80 g each) were ground in a Perten 3100 laboratory mill (Perten Instruments AB, Huddinge, Sweden) fitted with a standard 0.8 mm sieve. (Note that the Perten 3100 laboratory mill is currently used for grinding samples for falling number determination, AACC International Approved Method 56–81B.²⁷) In Approved Method 56–70, sedimentation volume is read after 20 min; here, sedimentation volume was recorded every 5 min for 30 min. All results are reported as specific sedimentation volume in mL g⁻¹ whole meal. SDS sedimentation method parameters were examined in three phases as follows.

Phase I variables: 10 and 15 g L⁻¹ SDS concentrations, sample weight and technician

Phase I employed the ten wheat samples described above in an otherwise 'standard' SDS sedimentation test as described in Approved Method 56–70 except that the SDS concentration and sample weight were varied: 10 and 15 g L⁻¹ SDS concentrations (20 and 30 g L⁻¹ stock solutions) and 3.15, 4.73 and 6.30 g sample weights. Samples were assayed in a completely factorial experiment; the entire Phase I design was performed by each of two independent technicians who were considered statistical 'blocks' in the analysis of variance (ANOVA; randomised complete block design). Sedimentation volumes were read every 5 min for 30 min.

Phase II variables: 5, 10, 15 and 20 g L⁻¹ SDS concentrations

Phase II was conducted by one technician and employed the same ten wheat samples used in Phase I (Table 1) in an otherwise 'standard' SDS sedimentation test as described in Approved Method 56–70 except that the range of SDS concentration (final) was expanded further to include 5, 10, 15 and 20 g L⁻¹ SDS. Sample weight was 6.30 g and sedimentation volumes were read every 5 min for 30 min.

Phase III variables: grinder and screen aperture (particle size)

Phase III employed the ten wheat samples described above in an otherwise 'standard' SDS sedimentation test except that, in addition to the Perten 3100 grinder, samples were also ground in a UDY Cyclone sample mill (UDY Corp., Fort Collins, CO, USA) fitted with 0.5 and 1.0 mm screens. All assays were conducted by one technician. Assays used 10 g L⁻¹ SDS final concentration and 6.30 g sample weight. Sedimentation volumes were read every 5 min for 30 min.

Statistical analysis

Data were analysed using ANOVA Proc Mixed (SAS v9.1, SAS Institute, Cary, NC, USA). The sedimentation volume of each replicated meal sample (subjected to a different test parameter such as detergent concentration, sample weight or grinder) was recorded over time. Time series measurements were considered repeated measures and were analysed using Proc Mixed to test for correlations among time points using various covariance models. Phases I and III used compound symmetry; Phase II used unstructured covariance. Type III sums of squares were used to calculate *F* values and test the relative significance of model components (main effects and interactions). Models for each of the three phases were analysed as complete factorial designs with all possible interaction terms, with the exception of the technicians in Phase I, who were considered blocks in a randomised complete block design. Sample least squares means were compared using standard *t* tests at a highly stringent *P* value of 0.0001.

RESULTS

Phase I examined the specific SDS sedimentation volume of ten diverse hexaploid wheat meals (Table 1) using the sole AACC International²⁷ SDS sedimentation protocol, Approved Method 56–70, which is for durum wheat and uses 30 g L⁻¹ SDS stock concentration (15 g L⁻¹ final) and 6.30 g of sample. Additionally, samples were assayed using 20 g L⁻¹ SDS stock concentration and with half (3.15 g) and three-quarter (4.73 g) sample weights. Two technicians each conducted the entire Phase I experiment. The sedimentation volume of samples was highly reproducible and replicate-to-replicate differences in reading the sedimentation volume averaged 0.675 mL (both technicians' data). (Note that the 100 mL graduated cylinders used here have 1 mL markings.) Depending on the sample weight, this difference translated into differences in specific volume of the order of 0.1–0.2 mL g⁻¹. All readings were converted to sedimentation volume per unit weight (g) of meal ('specific volume') and analysed using ANOVA Proc Mixed for mixed models involving repeated measures (time series) (Table 2). As noted with the small

replicate-to-replicate differences, the SDS sedimentation test was highly reproducible, with a small error variance. The range (0.985–0.989) of overall model R^2 for Phase I analysed for each time point indicated very high model fits.

The results of the mixed model ANOVA using time as repeated measures (Table 2) indicated that several of the model components were very highly significant, some with large F values. For this reason, the usual convention of assigning significance at $P \leq 0.05$

Table 2. Analysis of variance of specific SDS sedimentation volumes of ten hexaploid wheat meal samples using 3.15, 4.73 and 6.30 g sample weights and 10 and 15 g L⁻¹ SDS concentrations. Volumes were recorded every 5 min for 30 min; the entire experiment was conducted by each of two technicians

Source	DF	F value	P value
<i>Main effects</i>			
Time (T)	5	6750	<0.0001
Sample (S)	9	520	<0.0001
Sample weight (SW)	2	13.2	<0.0001
SDS concentration (SDS)	1	3.45	0.068
<i>Two-way interactions</i>			
T × S	45	172	<0.0001
T × SW	10	4.10	<0.0001
T × SDS	5	13.0	<0.0001
S × SW	18	3.70	<0.0001
S × SDS	9	0.22	0.99
SW × DS	2	9.24	0.0003
<i>Three-way interactions</i>			
T × S × SW	90	20.6	<0.0001
T × S × SDS	45	1.44	0.033
T × SW × SDS	10	5.27	<0.0001
S × SW × SDS	18	1.14	0.34
<i>Four-way interaction</i>			
T × S × SW × SDS	90	4.03	<0.0001

was not used. Instead, the relative magnitudes of the F values were considered along with the mean differences in sedimentation volumes associated with a particular effect.

Of the Phase I model main effects, time and sample had the largest F values (6750 and 520 respectively). Sample weight, while being significant and next in rank, had a small F value compared with time and sample. SDS concentration had no significant effect. Significant differences were expected *a priori* among the various time points, since the hydrated meal settled during the 30 min assay and volumes were expected to change (the rate of settling is further discussed below). Beyond this, clearly the most significant source of variation was among the wheat samples themselves. The F value for sample weight indicated that the absolute amount of sample had some small influence on the sedimentation volume.

Of the two-way model interaction terms, time interacting with sample had by far the greatest F value (Table 2). Next in rank was time × SDS concentration, followed by sample weight × SDS concentration. The F values for the three- and four-way model interaction terms were generally small, the largest being for time × sample × sample weight (F value 20.6). Although considered a random effect in the ANOVA, the two technician means were 8.65 and 9.75 mL g⁻¹, a difference about five- to tenfold larger than replicate differences within technician. When analysed as a fixed effect, the F value for technician was 1290 (data not shown).

The greatest single source of variation in the model was the differences in sedimentation volumes over time, and the interaction of time with sample was noteworthy (F value 172). Time interacting with SDS concentration and a three-way interaction with sample and sample weight were much smaller (Table 2).

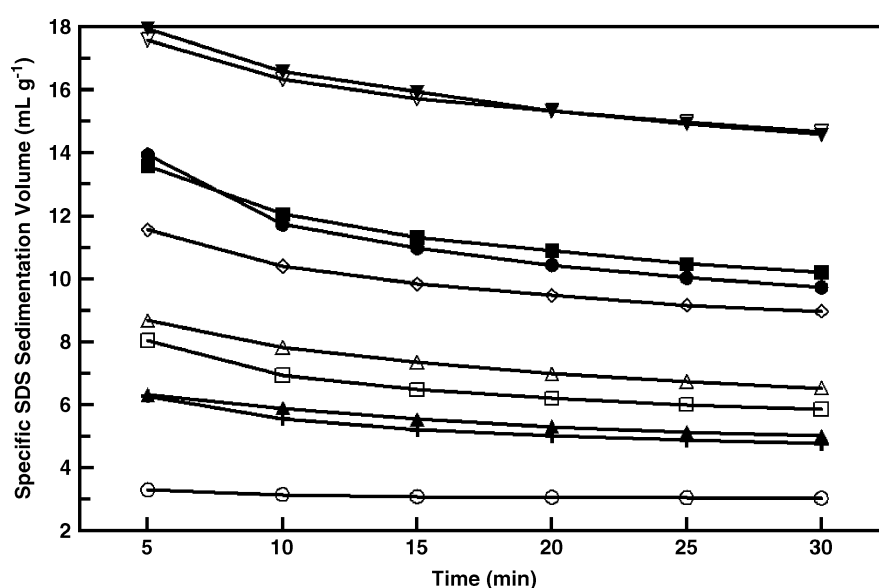


Figure 1. Specific SDS sedimentation volume of ten hexaploid wheat varietal samples averaged over two replications, two technicians, three sample weights and two SDS concentrations. Plot shows the changes in specific sedimentation volume over time. Symbols: ○, Hiller Bickleton; +, Eltan Bickleton; ▲, Estica Bickleton; □, Eltan Pullman; △, Estica Pullman; ◇, Estica Lind; ●, Finley Bickleton; ■, Finley Pullman; ▼, EST03005 Lind; ▽, Finley Lind.

Before returning to the main effects, both the time main effect and the largest of the interactions (time \times sample) were examined in greater detail. Figure 1 shows the source of both the time main effect and the time \times sample interaction, the latter due to some slight non-parallel slopes, especially between 5 and 10 min.

Returning to the ANOVA main effects, Fig. 1 provides the change in specific volume of the ten wheat samples over time. Although the zero time point is not presented, these volumes would necessarily represent slightly more than 100 mL (100 mL solution plus meal), equivalent to 16 and 32 mL g⁻¹ specific volumes (6.30 and 3.15 g sample weights respectively). After 5 min, high-sedimentation-volume samples such as Finley (from Lind) and EST03005 were still in the 95–100 mL volume range with 6.30 g sample weight, and 65–68 mL with 3.15 g, which produced mean specific volumes of the order of 18 mL g⁻¹. In contrast, the Hiller Club wheat sample had already settled to 3.3 mL g⁻¹ (equivalent to about 10 and 20 mL volumes at 3.15 and 6.30 g respectively). Decreases in specific volume from 5 to 30 min (i.e. settling) ranged from 0.3 mL g⁻¹ (Hiller) to 4.2 mL g⁻¹ (Finley from Bickleton). From 10 to 30 min, decreases in specific volume ranged from 0.1 mL g⁻¹ (Hiller) to 2.0 mL g⁻¹ (Finley from Lind and Bickleton). Clearly, large differences existed among the wheat samples for specific SDS sedimentation volume, and these differences were produced during the hydrating and mixing regimen. Further, the volume of the meals hydrated and swollen by the SDS/lactic acid/water solution changed only moderately after 10 min at 1 \times g.

Continuing the examination of the main effects, least squares mean specific volumes for sample weights were 8.80, 9.57 and 9.25 mL g⁻¹ (3.15, 4.73 and 6.30 g sample weights respectively). Least squares mean specific volumes for SDS concentrations were 9.32 and 9.09 mL g⁻¹ (10 and 15 g L⁻¹ SDS respectively). The relative magnitude of *F* values for these main effects was small and therefore they were not considered important. ANOVAs for each of the individual time points confirmed this result (data not shown).

Again, excluding the changes across time, by far the most important source of variation came from differences among the wheat samples. Given the preceding results of ANOVA, including both the large *F* value for sample and the relatively inconsequential interaction terms involving sample, the sample least squares means across all other variables were compared using *t* tests at *P* = 0.0001 (Table 3; see also Table 1). Specific sedimentation volumes of samples were well separated. As seen previously, the Finley HRW sample with 145 g kg⁻¹ protein (from Lind) and the EST030005 HRS sample with 151 g kg⁻¹ protein (also from Lind) produced the greatest specific sedimentation volumes, which were not significantly different. Lower-protein samples of Finley (from Bickleton and Pullman, 94 and

Table 3. Specific SDS sedimentation volumes^a of whole meal hexaploid wheat samples using AACC International Approved Method 56–70, SDS sedimentation test for durum wheat

Sample	Location	Specific volume (mL g ⁻¹)
Finley	Lind	15.88a
EST03005	Lind	15.78a
Finley	Pullman	11.43b
Finley	Bickleton	11.15b
Estica	Lind	9.91c
Estica	Pullman	7.36d
Eltan	Pullman	6.58de
Estica	Bickleton	5.54ef
Eltan	Bickleton	5.27f
Hiller	Bickleton	3.10g

^a Values are least squares means across two technicians, 5, 10, 15, 20, 25 and 30 min time points, 3.15, 4.73 and 6.30 g sample weights and 10 and 13 g L⁻¹ SDS concentrations. Means followed by the same letter are not significantly different at *P* = 0.0001.

100 g kg⁻¹ protein respectively) were next highest and similar in specific volume. Next in rank was Estica HRW at 129 g kg⁻¹ protein. Next lower in specific volume was a group of four samples beginning with Estica (from Pullman) with 90 g kg⁻¹ protein, Eltan SWW (from Pullman) also with 90 g kg⁻¹ protein, Estica (from Bickleton) with 80 g kg⁻¹ protein and Eltan (from Bickleton) with 77 g kg⁻¹ protein. Last in rank was a sample of the Club wheat Hiller also from Bickleton but with somewhat higher protein, 87 g kg⁻¹.

To examine these data in a different way, specific sedimentation volumes were plotted against protein content (Fig. 2). Although the data were limited, there was a tendency within Finley, Estica and Eltan varieties to increase in specific volume as protein content increases. For those three varieties that had two or more samples, the slopes were more or less similar. Although the data were not extensive enough to warrant further analysis, it is of interest to make note of the high-molecular-weight (HMW) glutenin subunits of these wheat samples (Table 1). The large differences in specific SDS sedimentation volumes between the hard red wheats (Finley and EST03005 *vs* Estica) and soft white wheats (Eltan *vs* Hiller) may be associated with the 5 + 10 *vs* 2 + 12 HMW subunit types. To explore this observation in more detail, further experiments are envisioned. In addition, the direct effect of the hardness locus^{28,29} has not been investigated but could be addressed using hard/soft near-isogenic lines.^{30–32} To highlight the inherent difference in protein quality between Finley and Estica, other samples of these two HRW wheat varieties from the same crop year and locations produced pan bread regression slopes of 87 and 54 cm³ per 10 g kg⁻¹ flour protein respectively (Engle DA, pers. comm.).

Phase II examined an expanded range of SDS concentration. It was often difficult to obtain a clear reading of sedimentation volume at 5 min with a number of the samples owing to the lack of a clear line of demarcation between sediment and

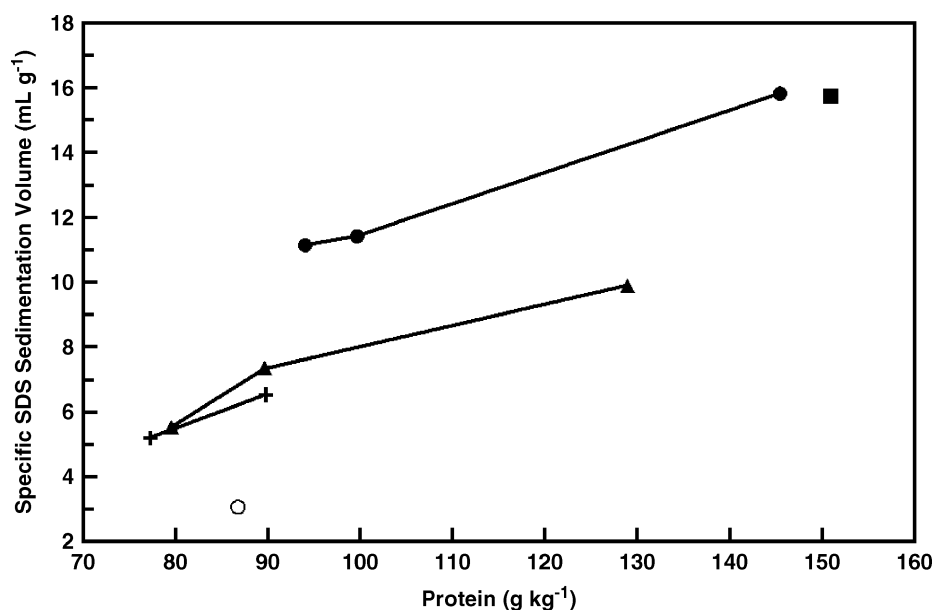


Figure 2. Specific SDS sedimentation volume of ten hexaploid wheat varietal samples averaged over two replications, two technicians, six sampling times, three sample weights and two SDS concentrations. Plot shows the specific volumes of each variety plotted against whole meal protein content. Symbols: ○, Hiller; +, Eltan; ▲, Estica; ●, Finley; ■, EST03005.

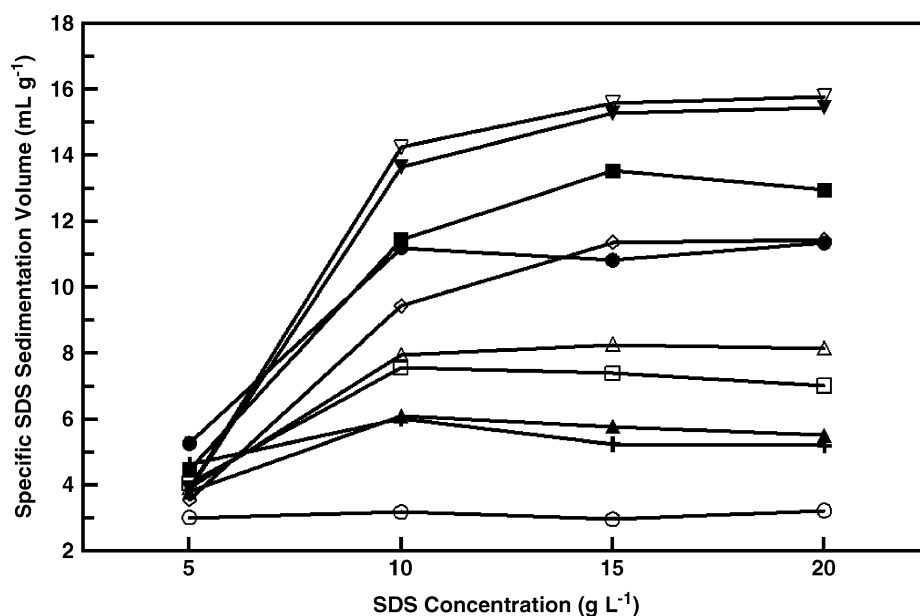


Figure 3. Specific SDS sedimentation volume of ten hexaploid wheat varietal samples averaged over two replications and five recording times. Plot shows the response of samples to changes in SDS concentration (final concentrations) (Phase II). Symbols: ○, Hiller Bickleton; +, Eltan Bickleton; ▲, Estica Bickleton; □, Eltan Pullman; △, Estica Pullman; ◇, Estica Lind; ●, Finley Bickleton; ■, Finley Pullman; ▼, EST03005 Lind; ▽, Finley Lind.

supernatant. Consequently, this time point was not included in the Phase II analysis. Mixed model ANOVA with time as repeated measures again produced exceptionally large *F* values for the main effects (7680 for recording time, 4440 for sample and 7390 for SDS concentration). Of the interaction terms, only sample \times SDS concentration (*F* value 402, the largest) was deemed worth considering further. Figure 3 highlights not only the large effect of sample and SDS concentration but also the source of the significant sample \times SDS concentration interaction. Clearly, 5 g L⁻¹ SDS concentration was insufficient to provide adequate swelling of wheat

meal constituents or develop inherent differences amongst these diverse hexaploid wheat samples. From 10 to 15 to 20 g L⁻¹ SDS concentrations, specific volumes changed little, especially for those samples with lower inherent sediment volumes. Of note, the specific sedimentation volume of the very-weak-gluten Hiller Club wheat variety changed little over the entire range of SDS concentration. Over all samples the least squares mean specific volumes were 4.05, 9.08, 9.63 and 9.61 mL g⁻¹ for 5, 10, 15 and 20 g L⁻¹ SDS concentrations respectively.

Phase III of the research examined the effects of grinder and screen aperture (particle size) on

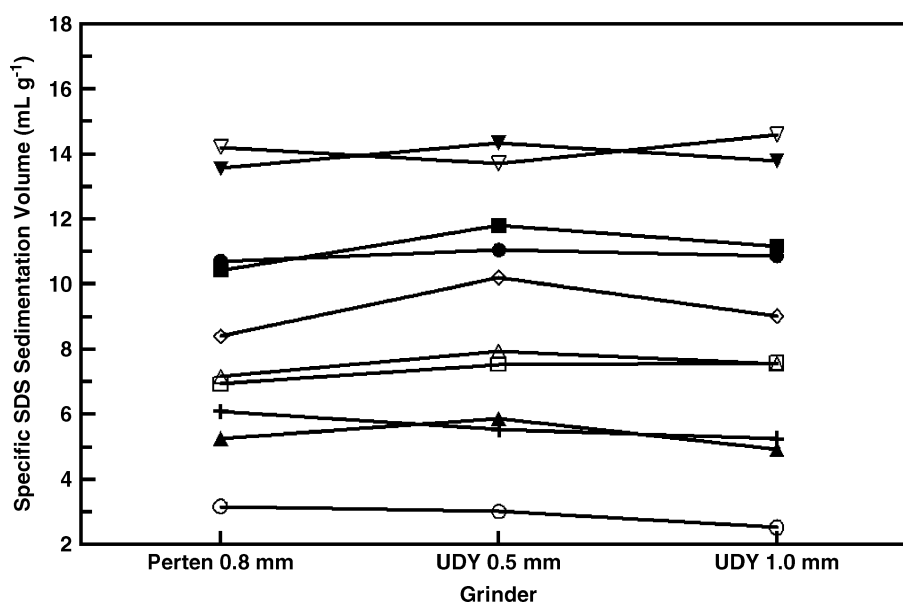


Figure 4. Specific SDS sedimentation volume of ten hexaploid wheat varietal samples averaged over two replications and five recording times. Plot shows the response of samples to changes in grinder type and screen aperture (particle size) (Phase III). Symbols: ○, Hiller Bickleton; +, Eltan Bickleton; ▲, Estica Bickleton; □, Eltan Pullman; △, Estica Pullman; ◇, Estica Lind; ●, Finley Bickleton; ■, Finley Pullman; ▼, EST03005 Lind; ▽, Finley Lind.

Table 4. Analysis of variance of specific SDS sedimentation volumes of ten hexaploid wheat meal samples prepared by grinding with the Perten 3100 grinder and the UDY Cyclone mill fitted with 0.5 and 1.0 mm aperture screens; volumes were recorded every 5 min for 30 min

Source	DF	F value	P value
<i>Main effects</i>			
Time (T)	5	10 900	<0.0001
Sample (S)	9	11 300	<0.0001
Grinder (G)	2	186	<0.0001
<i>Two-way interactions</i>			
T × S	45	215	<0.0001
T × G	10	43.8	<0.0001
S × G	18	54.0	<0.0001
<i>Three-way interaction</i>			
T × S × G	90	14.6	<0.0001

specific SDS sedimentation volume. The results of the ANOVA with time as repeated measures are presented in Table 4. Recording time (F value 10 900) and sample (F value 11 300) were by far the most significant sources of variation. Grinder had an F value of 186. Time \times sample was again the largest interaction (cf. Table 2), followed by sample \times grinder. The other two- and three-way model interaction terms were not considered further. Figure 4 illustrates the grinder effect and the small sample \times grinder interaction. The wheat samples appeared to fall into six discrete groups: four 'pairs' and two 'singles'. Samples of a pair were considered to have similar SDS sedimentation volume characteristics, such that pairs would change in rank order among the three grinders but were never observed to 'leave' a group and cross over into another group. Similarly, single-group samples

remained distinct and did not change in rank order among the ten samples. The overall grand least squares means for the three grinders were 8.60, 9.10 and 8.73 mL g⁻¹ for the Perten 3100, UDY 0.5 mm and UDY 1.0 mm screen respectively. These three least squares means were significantly different as determined by paired t tests performed under the mixed model covariance ANOVA. Although each grinder produced somewhat different specific SDS sedimentation volumes, the differences were relatively minor compared with the differences among the wheat samples. Consequently, there appeared to be no evidence for a strong particle size affect.

DISCUSSION

The physicochemical basis for wheat sedimentation tests is the differential swelling and flocculation of glutenin and other insoluble constituents of the wheat grain and their interaction with water at acid pH and, optionally, SDS. In this regard the addition of the ionic detergent SDS to the acidified water system of Zeleny¹ increased two- to four-fold the electrostatic repulsion, swelling and flocculation of charged glutenins and flour particles.^{33,34} The present study shows that the SDS sedimentation test for durum wheat, AACC International Approved Method 56–70, which was patterned after Axford *et al.*,^{8,9} is highly sensitive to differences amongst hexaploid 'bread' wheat samples (Tables 2–4, Figs. 1–4). We selected a diverse set of ten hexaploid wheat samples *a priori* based on gluten strength, protein content and grain hardness to represent a range of gluten strength and quantity as would be encountered in traditional wheat breeding programmes and in world commerce. Replicate assays were highly reproducible and ANOVA indicated very

small error variances with large *F* values. We found a consistent, albeit relatively minor, difference between two skilled technicians who conducted Phase I.

The great number of modifications and variations to the original method of Axford *et al.*,^{8,9} itself a modification of the method of Zeleny,¹ highlights the versatility and adaptability of the general sedimentation procedure. SDS sedimentation has been applied to durum and hexaploid wheat samples and is especially useful in breeding populations where grain quantity is limiting. In this regard, whole meals (which are more convenient to prepare) have been shown to perform similarly to milled flours.^{8,9,15,16,22} Further, to be market-applicable in commercial channels, an automated SDS sedimentation test would preferably use whole-grain meals so as to obviate the need to produce flour. A further advantage would ensue if an existing sample preparation step, such as grinding a grain aliquot for the falling number test, could be utilised in an automated sedimentation test. Results of the present study indicate that variance in grinder or particle size has a minor effect on specific sedimentation volume.

The rate of sedimentation in SDS/LA is fairly rapid, such that most settling has taken place by 5 min and little further settling occurs by 10 min (Table 2, Fig. 1). The rate of settling during this early time period (0–5 min) is actually inversely related to the final sediment volume, i.e. the rate for Hiller Club wheat was by far the greatest because it reached its very low volume before 5 min had elapsed. After 10 min there was essentially no change in ranking of samples for sedimentation volume. The results suggest that optical monitoring devices could likely discern sedimentation velocity differences among samples before the eye could delineate a demarcation between supernatant and sediment.

Protein content is well known to influence SDS sedimentation volume; however, as mentioned earlier, the influence is variety-dependent and reflective of the protein quality (see e.g. Refs 18 and 22). Krattiger and Law¹⁹ sought to minimise or eliminate this effect. Conversely, it could be considered beneficial that the SDS sedimentation test ‘captures’ both sources of variation: protein quality and quantity. Certainly from a practical standpoint, protein content is easily measured and could provide an additional factor upon which to interpret a given specific SDS sedimentation volume. Figure 2 indicates that different wheat varieties may possess different inherent intercepts that relate to intrinsic properties of the glutenin or other grain constituents. This observation will require more research to be substantiated. Carter *et al.*²² showed that different soft wheat varieties exhibited different responses (slopes) to changes in protein content.

Sediment volume generally increases with SDS concentration until reaching a threshold concentration of about 10–15 g L⁻¹ SDS (Fig. 3).^{15–18} Since the response is due to inherent differences among wheat

samples, it is not consistent from variety to variety (Fig. 3). The primary consideration appears to be to supply sufficient SDS to accommodate high-protein/strong-gluten samples.¹⁸ The Club wheat sample Hiller appeared to have a very low requirement for SDS.

The AACC collaborative study for Approved Method 56–70¹⁷ found that the considerable inter-laboratory variation was mostly attributable to differences in grinders and grinder feeding rates. As noted above, our data indicated that there were no marked effects for either grinder type or screen aperture (and ostensibly particle size distribution) (Fig. 4). This discrepancy may be due to the differences in kernel texture between durum and hexaploid wheat samples.

In conclusion, the SDS sedimentation assay of Axford *et al.*^{8,9} as embodied by AACC International Approved Method 56–70 for durum wheat is a robust, highly reproducible assay and can well delineate soft and hard hexaploid bread wheat samples based on protein quality and quantity. Sample weight (if corrected to unit weight basis), SDS stock concentration of at least 10 g L⁻¹, grinder type and screen aperture were minor sources of variation in SDS sedimentation volume relative to the effects due to differences among wheat samples. Owing to the settling of the sediment over time, the recording time was a large statistical source of variation. However, interactions with time were relatively small, such that a recording time of at least 10 min or more consistently ranked the wheat samples.

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